

CLAIMS

1. A method of obtaining an increased yield of biomass of a lactic acid bacterial cell culture, the yield exceeding that which can be obtained at maximum from substrate level
5 phosphorylation, the method comprising the steps of
 - (i) providing in the cells of the culture conditions that results in a reduced glycolytic flux, and
 - 10 (ii) providing conditions that enable the cells under aerobic conditions to have a respiratory metabolism.
2. A method according to claim 1 wherein the increased biomass is obtained by an increased yield of ATP in the cells.
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3. A method according to claim 2 wherein the increased yield of ATP is obtained by activating the native ATP synthase activity or enhancing the expression of the ATP synthase of the cell.
- 20 4. A method according to claim 3 wherein the ATP synthase activity of the cells is activated by increasing the proton gradient of the cells and/or reducing the ATP/ADP ratio.
5. A method according to claim 4 wherein the reduced ATP/ADP ratio is provided by the reduced glycolytic flux in step (i).
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6. A method according to claims 1 to 5 wherein the reduced glycolytic flux in the cells is provided by cultivating the culture under carbon source limiting conditions.
7. A method according to claim 6 wherein the carbon source limitation is provided by
30 using a growth-limiting concentration of the carbon source.
8. A method according to claims 6 or 7 wherein the carbon source limitation is provided by cultivating the cells under fed batch and/or continuous conditions.

9. A method according to claim 6 wherein carbon source limiting conditions are provided by using a carbon source which is not readily metabolised by the cell.

10. A method according to claim 9 wherein the carbon source is selected from the group consisting of ribose, xylose, arabinose, allose, mannose, gulose, idose, galactose, talose and maltose.

11. A method according to claim 6 wherein the carbon source limiting conditions are provided by modifying the cells in order to assimilate the carbon source at a lower rate relative to its parent strain.

12. A method according to claim 3 to 11 wherein the increased yield of ATP is provided by increasing the expression of ATP synthase.

13. A method according to claim 12 wherein the expression of ATP synthase is increased by introducing in the cells an increased number of copies of a gene or genes expressing ATP synthase activity.

14. A method according to claim 12 wherein the expression of ATP synthase is increased by inserting a regulatory sequence that enhances expression or by reducing or inhibiting inhibition of the expression of ATP synthase.

15. A method according to any of claims 3-14 wherein the ATP synthase activity of the cells is activated by increasing the proton gradient of the cell.

16. A method according to claim 15 wherein the proton gradient is increased by increasing the expression of the native components of the electron transport chain.

17. A method according to claim 15 wherein the proton gradient is increased by increasing expression of NADH dehydrogenase or by reducing or eliminating the expression of a NAD⁺ regenerating enzyme activity.

18. A method according to claim 17 wherein the NAD⁺ regenerating enzyme activity is NADH oxidase activity.

19. A method according to claim 15 wherein the proton gradient is increased by increasing the expression of the endogenous cytochromes including cytochrome bd.
20. A method according to claim 15 wherein the proton gradient is increased by
5 introducing a heterologous respiratory chain component.
21. A method according to claim 20 wherein the component is a cytochrome selected from the group consisting of cytochrome type bo, cytochrome type aa₃ and cytochrome complex cyt bc₁.
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22. A method according to claims 1 to 21 wherein the conditions that enables the cell under aerobic conditions to have a respiratory metabolism are provided by cultivating the cells in a medium containing a quinone and/or a porphyrin compound or by cultivating the cells under conditions which favour the formation of a quinone, a porphyrin compound
15 and/or a cytochrome.
23. A method according to any of claims 1-22 wherein the biomass comprises cells comprising a gene coding for a desired gene product.
- 20 24. A method according to claim 23 wherein the expression of the gene is under the control of an inducible or a constitutive promoter.
25. A method according to claim 23 wherein the desired gene product that is encoded is selected from the group consisting of an enzyme and a pharmaceutically active gene
25 product.
26. A method according to claim 25 wherein the enzyme gene product is a milk clotting enzyme.
- 30 27. A method according to claims 1 to 26 wherein the cell is of a lactic acid bacterial species selected from the group consisting of a *Lactococcus* species, a *Streptococcus* species, a *Leuconostoc* species, a *Lactobacillus* species and an *Oenococcus* species.
28. A method according to any of claims 1-27 wherein the resulting cell biomass is a lactic
35 acid bacterial food or feed starter culture.

29. A method of reducing the content of by-products in a production of biomass of lactic acid bacterial cells said method comprising of a step of increasing the yield of biomass by (i) providing in the cell conditions that results in a reduced glycolytic flux and (ii) providing
5 conditions that enables the cell under aerobic conditions to have a respiratory metabolism.

30. A lactic acid bacterial cell obtainable by the method according to claims 1 to 28.

10 31. A lactic acid bacterial cell produced by culturing the cell under conditions that results in a reduced glycolytic flux, and under conditions that enable the cells to have, under aerobic conditions, a respiratory metabolism, said cell having, relative to a lactic acid bacterial cell produced in the presence of a readily metabolised carbon source in excess, an increased activity of the enzymes involved in the uptake and/or degradation of a that
15 carbon source in which the bacterial cell has been propagated, and containing a detectable amount of a porphyrin compound and/or a cytochrome.

32. A lactic acid bacterial cell according to claim 31 which constitutively expresses the *lac* operon and/or *gal* operon.

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33. A lactic acid bacterial cell according to claim 32 wherein constitutive expression is provided by a mutation in the gene coding for the *lac* repressor and/or *lac* operator.

34. A lactic acid bacterial cell according to claim 31 that contains at least 0.1 ppm on a dry
25 matter basis of a porphyrin compound.

35. A lactic acid bacterial cell according to claim 31 that contains at least 0.1 ppm on a dry matter basis of a cytochrome.

30 36. A lactic acid bacterial cell according to claim 31 which is a cell of a lactic acid bacterial species selected from the group consisting of a *Lactococcus* species, a *Streptococcus* species, a *Leuconostoc* species, a *Lactobacillus* species and an *Oenococcus* species.

37. A starter culture composition comprising the lactic acid bacterial culture according to
35 claim 1 or a lactic acid bacterial cell according to claims 30 or 31.

38. A composition according to claim 37 where the composition is in the form of a frozen, liquid or freeze-dried composition.

5 39. A composition according to claims 37 or 38 containing an amount of viable culturally modified lactic acid bacterial cells which is in the range of 10^4 to 10^{12} CFU per g.

40. A composition according to claims 37 to 39 that comprises cells of two or more different lactic acid bacterial strains.

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41. A composition according to claims 37 to 40 which further comprises at least one component enhancing the viability of the bacterial cell during storage, including a bacterial nutrient and/or a cryoprotectant.

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